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(Research Article)

Histomorphometric Analysis of Cardiopulmonary and Renal Organs in Philippine Native Darag and Commercial Redbro Chickens

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Abstract

Background: Philippine native Darag chickens are widely distributed, locally adapted, and valued for preferred flavor and perceived hardiness despite slower growth, smaller body size, and leaner musculature than commercial broilers. However, baseline histomorphometric data on major organs of this breed remain limited. This study evaluated the developmental histomorphometry of the heart, lungs, and kidneys of Darag chickens compared with Redbro commercial hybrid broilers, selected because of their roles in oxygen delivery, gas exchange, and metabolic waste elimination, respectively, and the scarcity of baseline data in Philippine native chickens. **Methods:** Heart, lung, and kidney tissues were collected from 100 day-old male Darag and 100 Redbro chicks. Three birds per strain were sampled at weeks 1, 2, 4, 6, and 8 for histologic processing and quantitative analysis. Associations among organ parameters, body weight, and feed intake were assessed. **Results:** Age significantly influenced heart muscle fiber thickness, parabronchial density, and parabronchial lumen diameter, while strain affected heart muscle fiber thickness and air capillary area fraction. Significant age–strain interactions were observed across all kidney parameters. Body weight and feed intake showed positive correlations with selected cardiopulmonary morphometric parameters across strains. **Conclusions:** Findings provide preliminary structural baseline data; larger functional studies are recommended.

Keywords

Darag; Philippine native chicken; Redbro; Heart; Lungs; Kidneys; Histomorphology

1. Introduction

Chicken meat ranks second to pork in Filipino diets. In 2016, the Philippines produced 1,674.50 metric tons of chicken, following pork at 2,231.66 metric tons [1]. In 2018, per capita chicken

consumption averaged 6.89 kg nationally and 12.94 kg in the National Capital Region [2]. These figures underscore the sector's importance and sustained consumer demand. Demand remains elevated, particularly following the African swine fever surge. Generally, meat-type broilers are more production-efficient than local breeds, whereas indigenous chickens offer disease resistance, environmental adaptability, and distinctive meat quality [3].

The Darag chicken originates from Western Visayas. It is central to Visayan cuisine, supporting regional popularity and market potential. Compared with the 28–45 days to market typical of commercial broiler-type chickens, Darag chickens undergo a 21–45-day "hardening" period to acclimate juveniles to environmental conditions. After hardening, birds are released to range and typically harvested at 75–120 days—approximately three times longer than broilers. Rural consumers value its dual-purpose traits and palatable meat, characterized by lower fat, higher unsaturated fatty acids, high protein, and notable potassium content [4, 5].

Philippine native chickens combine resilience, favorable sensory attributes, and sustainability. Adaptation to local conditions reduces disease risk and reliance on chemicals. Free-range systems promote welfare, and slower growth may yield nutritionally favorable meat for consumers seeking natural products.

The heart, lungs, and kidneys were selected as target organs owing to their central roles in oxygen delivery, gas exchange, and metabolic waste elimination, respectively, and because histomorphometric baseline data for these organs in Philippine native chicken strains are lacking in the published literature. The present study evaluated histomorphometric differences in these organs between Darag chickens and Redbro commercial broiler-type chickens.

2. Materials and Methods

2.1. Birds and Management

One hundred day-old male Philippine Darag chicks and 100 Redbro commercial hybrid broiler-type chicks were obtained from the Genome-Wide Association Study Project of the Institute of Animal Science, College of Agriculture and Food Sciences, University of the Philippines Los Baños. The birds were housed at the Institute of Animal Science Experimental Poultry Farm for up to 16 weeks during November 2022–January 2023, or until Darag chickens reached 1 kg. Birds at weeks 1, 2, 4, 6, and 8 were sampled.

Each pen measured 3 ft (length) × 4 ft (width) × 2.5 ft (height) and housed 10 birds. Pens were maintained under 24-hour light at 31 °C for the first 21 days, then under 12-hour light at 28 °C thereafter. Animals had ad libitum access to clean water and received booster mash for brooding (0–21 days) and starter feed for early growth (22–56 days). For disease prevention, birds received two Newcastle disease vaccines: B1B1 on day 7 and La Sota P9 on day 14.

All procedures were approved by the University of the Philippines Los Baños Institutional Animal Care and Use Committee (approval number UPLB-2023-036).

2.2. Experimental Design

Two sets of 100 male day-old chicks were used (one set Darag, one set Redbro). Both sets were raised under the same husbandry conditions described above. Random sampling was conducted at weeks 1, 2, 4, 6, and 8. At each time point, three birds per group were sampled; at week 2, six birds per group were available and included. These sampling intervals were selected to capture early, rapid, and intermediate growth phases across the study period.

2.3. Sample Collection

Birds were randomly selected and euthanized by decapitation on prescheduled sampling days. Standard chicken necropsy procedures were followed: (1) the abdominal cavity was opened by midline incision and the abdominal wall reflected; (2) the entire gastrointestinal tract, liver, and spleen were removed; (3) the kidneys were bluntly dissected free from the vertebral column; (4) the lungs were separated from the ribs by careful blunt dissection and removed from the thorax; and (5) the heart was excised by severing its major vascular attachments. All organs were weighed and immediately immersed in neutral buffered formalin for a minimum of 72 hours.

2.4. Tissue Processing and Histological Examination

Histological processing followed a standard paraffin-embedding protocol in sequential order. After fixation, tissues were rinsed in running tap water. Dehydration was performed through a graded ethanol series (70%, 80%, 90%, 100%) with two changes at each concentration. Tissues were cleared in two changes of 100% xylene and infiltrated with paraffin wax prior to embedding. Paraffin blocks were sectioned at 5 µm on a rotary microtome; one section of every four was selected for staining.

For hematoxylin and eosin (H&E) staining, sections were deparaffinized in four changes of xylene (5 minutes each) and rehydrated through a descending ethanol series (100%, 90%, 80%, 70%). Slides were rinsed in running tap water for 5 minutes and stained with hematoxylin for 2 minutes. After bluing in tap water, slides were soaked five times in 80% ethanol, stained with eosin for approximately 5–10 minutes, and dehydrated through ascending ethanol concentrations. Before mounting, slides were cleared in two changes of xylene (5 minutes each), mounted with a drop of resinous mounting medium, and coverslipped.

Stained slides were examined under a compound light microscope (AmScope, China) to assess histomorphometric parameters of the heart, lungs, and kidneys across time points.

2.5. Histomorphometric Examination

All morphometric measurements were performed using Fiji (ImageJ, National Institutes of Health, Maryland, USA) by a single trained observer using standardized protocols.

Heart. Left and right ventricular wall thicknesses were measured at five locations on transverse sections at 4× magnification. For larger samples, Adobe Photoshop Photomerge was used to stitch separately captured images. Muscle fiber thickness was measured in the free wall of the left ventricle. Five fibers per animal were measured at their narrowest transverse diameter, perpendicular to the long axis of the fiber. Boundaries between adjacent fibers were demarcated where the sarcolemma of one fiber was visually distinct from that of the adjacent fiber. Cross-sections were selected to provide a consistent, reproducible fiber profile.

Lungs. Parabronchial density was quantified as the number of parabronchi per microscopic field (five fields per animal) at 4× magnification. Parabronchial lumen diameter was measured from cross-sectional profiles of five parabronchi per animal; the internal diameter was taken at the widest point of each cross-section. Air capillary area fraction was estimated by color-contrasting image analysis (blood capillaries assigned black; air capillaries assigned white) and expressed as the percentage of the tissue cross-sectional area occupied by air capillaries.

Kidneys. Renal corpuscle density was determined as the number of corpuscles in one intact kidney lobule at 10× magnification. Renal corpuscle diameter was measured as the internal lumen diameter of five corpuscles per animal. Proximal and distal convoluted tubules were differentiated based on standard H&E histological criteria: proximal convoluted tubules (PCT) were identified by their larger lumen, taller cuboidal-to-columnar epithelium with abundant eosinophilic cytoplasm and indistinct apical brush border; distal convoluted tubules (DCT) had a smaller, rounder lumen, lower cuboidal epithelium, and paler, less eosinophilic cytoplasm. Tubule densities were counted in five fields per animal at 10× magnification.

2.6. Statistical Analysis

Data were analyzed in Prism (GraphPad Software, San Diego, CA, USA). A mixed-effects model was applied to accommodate missing values and to test the effects of age, strain, and their interaction on cardiopulmonary and renal parameters. Significance thresholds were set at $P < 0.05$ (significant), $P < 0.01$ (highly significant), and $P < 0.001$ (very significant). Given that three animals were sampled per group at each time point, the present study is best interpreted as exploratory; statistical findings indicate trends that warrant confirmation in larger cohorts.

A two-tailed Pearson correlation coefficient was used to assess relationships between measured parameters, body weight (BW), and total feed consumption, applying the same significance thresholds.

3. Results

3.1. Left Ventricular Wall Thickness

Left ventricular wall thickness did not differ significantly across weekly measurements in either the Darag or Redbro strains. Temporal fluctuations were evident (Figure 1D); these irregular patterns likely reflect high intra-group biological variability given the small sample size. In the Darag group, values rose from week 1 to 2, declined at week 4, and increased again from week 6 to 8. In the Redbro group, values increased steadily from weeks 1–4, decreased at week 6, and rose slightly at week 8.

(B) Whole heart. (C) Left ventricular wall at 40× magnification; representative measurement of

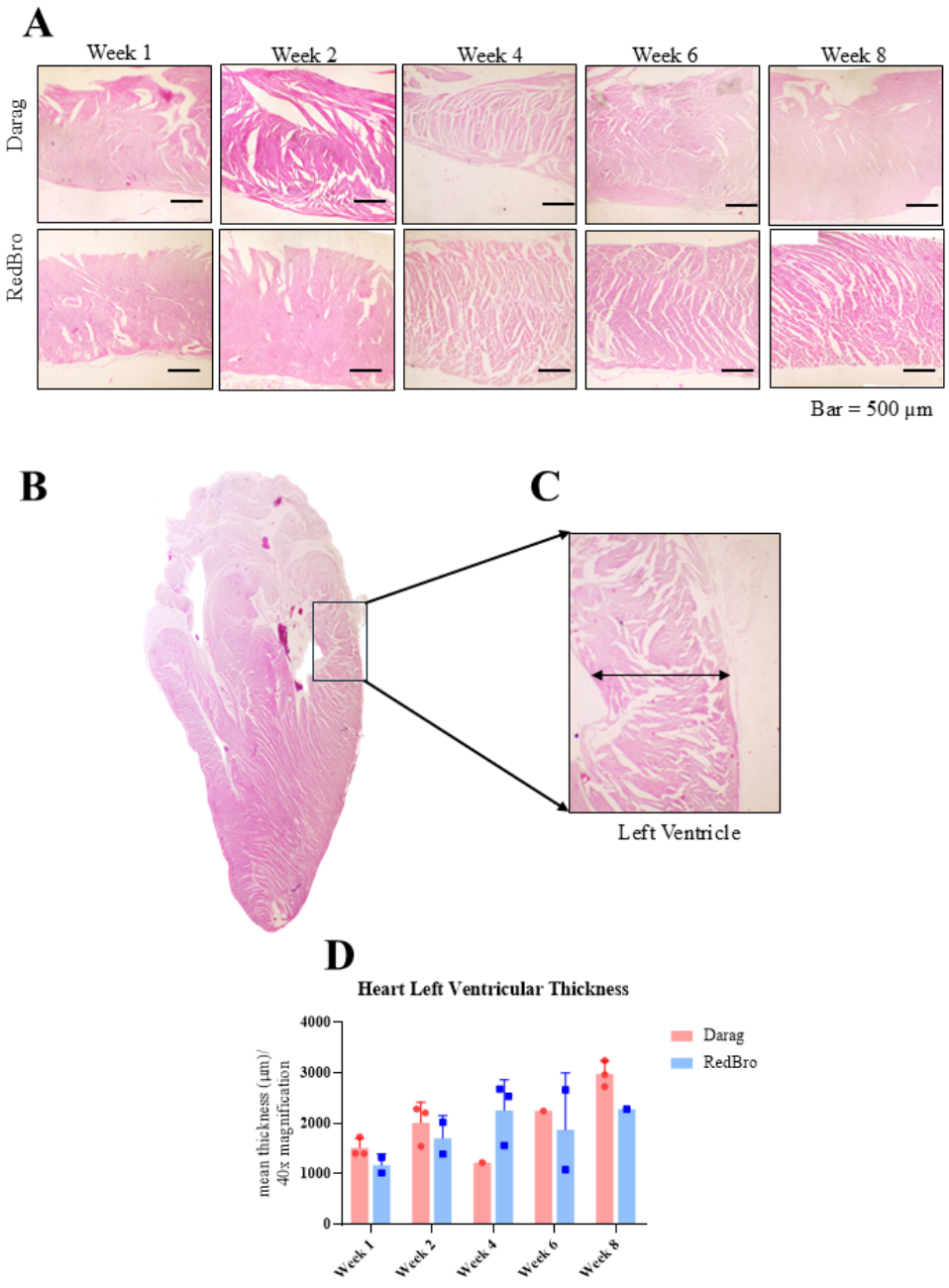

Figure 1. Left ventricular wall thickness did not differ significantly between Darag and Redbro. (A) Representative images of the left ventricular wall at weeks 1, 2, 4, 6, and 8. Scale bar = 500 µm.

thickness indicated by double-headed arrows. (D) Mean left ventricular wall thickness. Data are expressed as mean ± SD from five tissue sections per animal (n = 3 animals per group).

3.2. Right Ventricular Wall Thickness

Right ventricular wall thickness showed no significant weekly differences between strains. The pattern was irregular (Figure 2D), consistent with high intra-group variability. In the Darag group, a small increase occurred from week 1 to 2, followed by a decrease at week 4 consistent with the representative image (Figure 2A), and an increase at week 6. In the Redbro group, values increased at weeks 1, 2, 4, and 6, then decreased slightly at week 8.

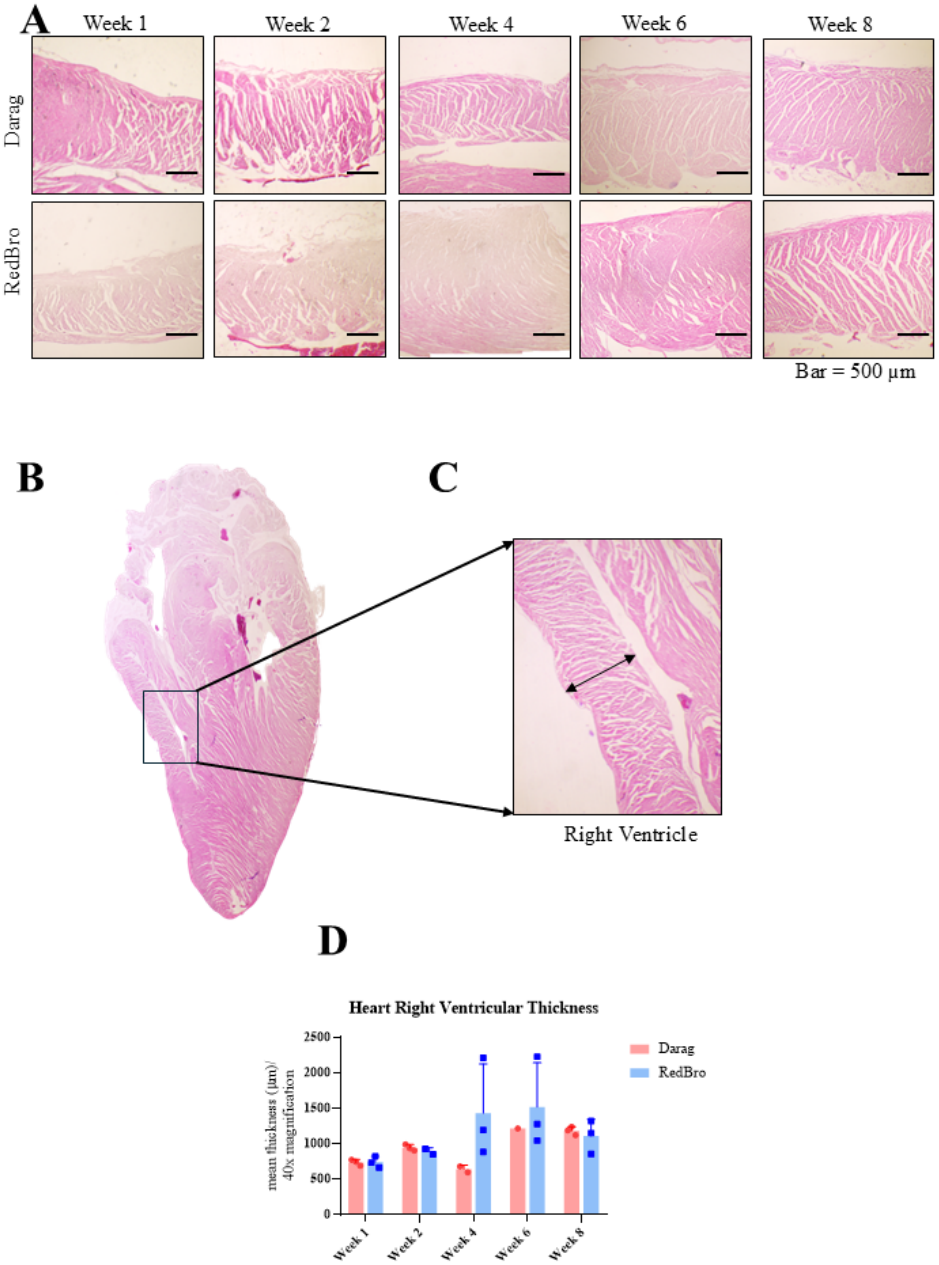

Figure 2. Right ventricular wall thickness did not differ significantly between Darag and Redbro. (A) Representative images of the right ventricular wall at weeks 1, 2, 4, 6, and 8. Scale bar = 500 µm.

(B) Whole heart. (C) Right ventricular wall at 40× magnification; representative measurement of thickness indicated by double-headed arrows. (D) Mean right ventricular wall thickness. Data are expressed as mean ± SD from five tissue sections per animal (n = 3 animals per group).

3.3. Muscle Fiber Thickness

Muscle fiber thickness in the left ventricular free wall differed significantly between strains at weeks 4 and 8, whereas no significant differences were observed at other time points. For both strains, values increased steadily from week 1 to week 8 (Figure 3B), with Redbro consistently demonstrating greater fiber thickness at the weeks where significant differences were observed.

3.4. Parabronchial Density

Parabronchial density did not differ significantly between strains. Both exhibited a

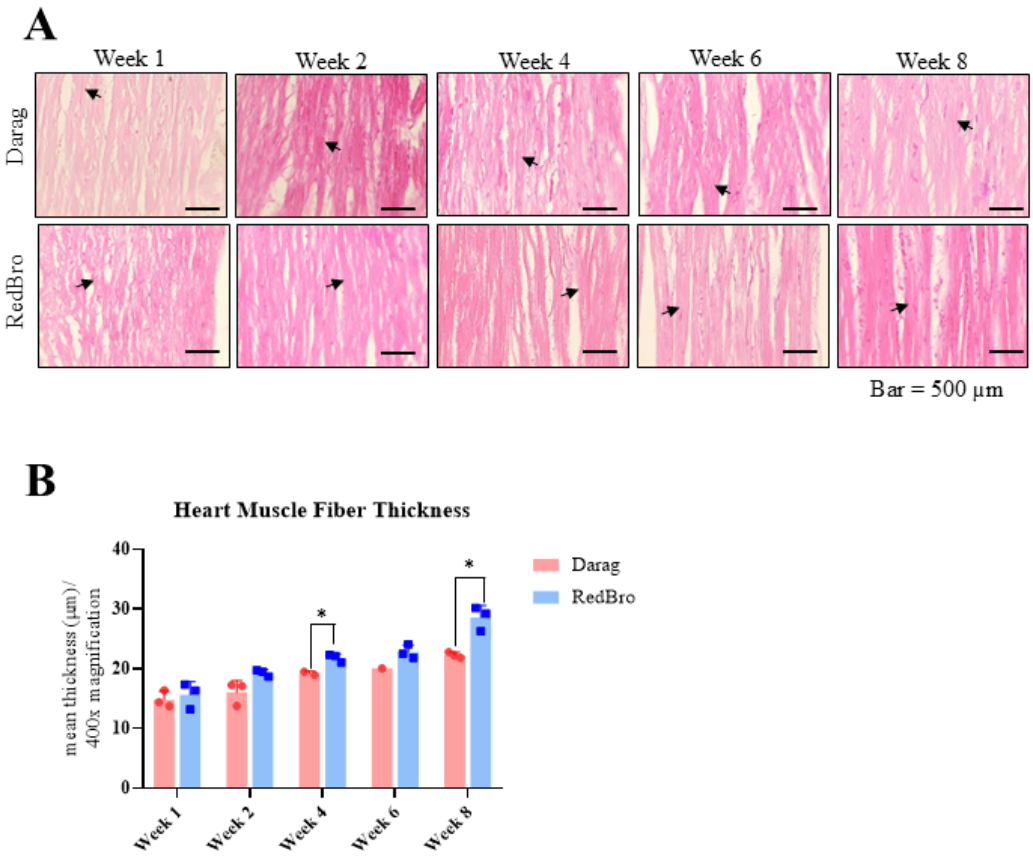

Figure 3. Heart muscle fiber thickness differed significantly between strains at weeks 4 and 8. (A) Representative images of left ventricular cardiomyocytes at weeks 1, 2, 4, 6, and 8; cardiac muscle fibers are labeled with arrows. Scale bar = 500 µm. (B) Mean muscle fiber thickness. Data are expressed as mean ± SD from five fibers per animal (n = 3 animals per group).

clear downward trend from week 1 to week 8 (Figure 4B). Representative images likewise illustrate a progressive decline in the number of parabronchi per field (Figure 4A).

(Figure 4C). In the Darag group, values increased from week 1 to week 6 and decreased slightly at week 8.

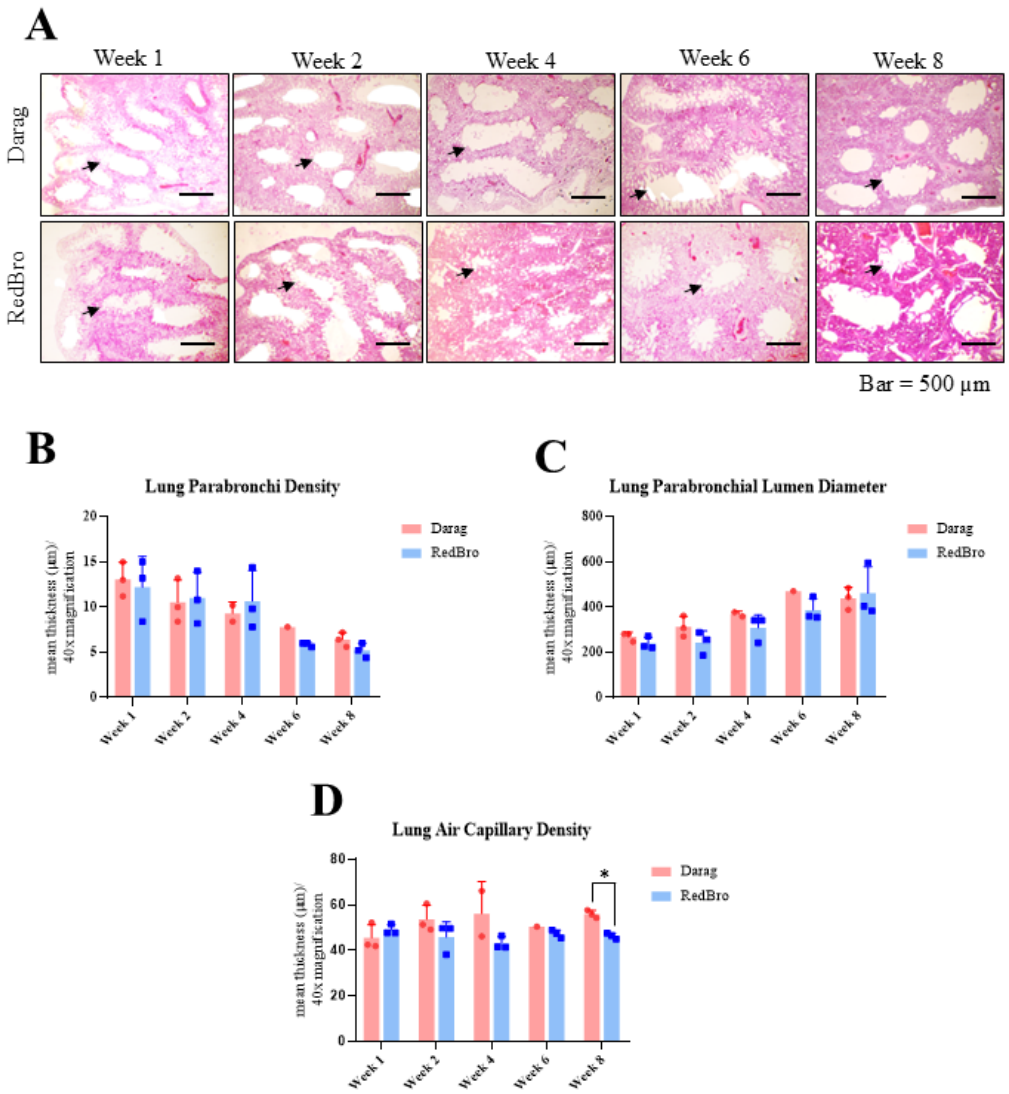

Figure 4. Lung air capillary area fraction differed significantly between strains at week 8, whereas parabronchial density and parabronchial lumen diameter did not. (A) Representative lung images at weeks 1, 2, 4, 6, and 8; parabronchi are labeled with arrows. Scale bar = 50 µm. (B) Mean lung parabronchial density. (C) Mean lung parabronchial lumen diameter. (D) Mean lung air capillary area fraction. Data are expressed as mean ± SD from five fields or structures per animal (n = 3 animals per group).

3.5. Parabronchial Lumen Diameter

Parabronchial lumen diameter did not differ significantly between strains. In the Redbro group, values increased steadily from week 1 to week 8

3.6. Air Capillary Area Fraction

Air capillary area fraction differed significantly between strains at week 8. The bar graph showed irregular patterns for both strains

(Figure 4D). In the Darag group, values increased slightly at weeks 1, 2, and 4, decreased at week 6, and increased again at week 8. In the Redbro group, values decreased from weeks 1–4, increased at week 6, and decreased slightly at week 8. One Redbro slide at week 8 showed processing artifacts and was excluded from analysis; this is acknowledged as a limitation.

(Figure 5). In the Darag group, counts were high at week 1, decreased sharply at week 2, and then fluctuated thereafter. In the Redbro group, counts varied but remained near the average of 10–20 corpuscles per week. Irregular trends in both strains likely reflect biological variability inherent to the small sample size.

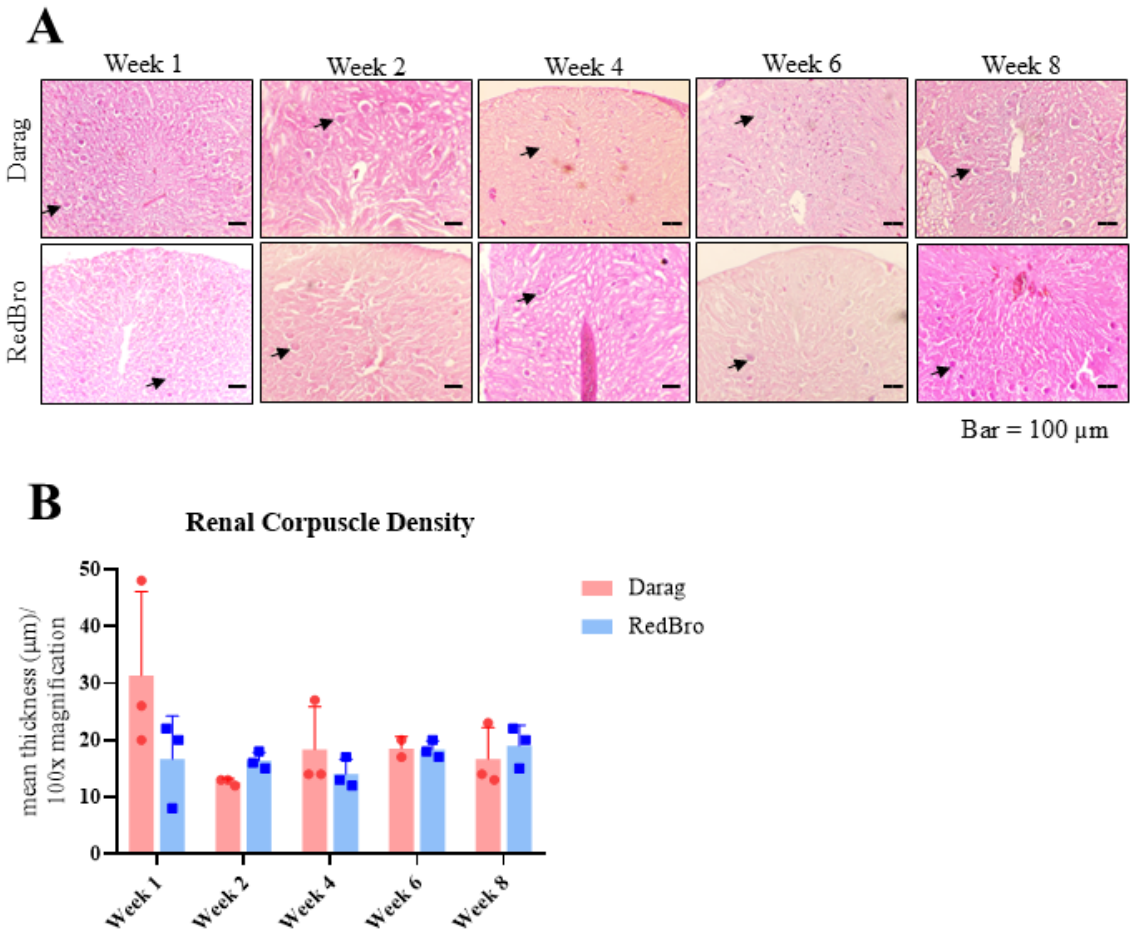

Figure 5. Histomorphology of renal corpuscle density in Darag and Redbro strains. No significant differences were observed between strains. (A) Representative kidney images at weeks 1, 2, 4, 6, and 8; mammalian-type renal corpuscles are labeled with arrows. Scale bar = 100 µm. (B) Bar graph showing mean renal corpuscle density. Data are mean ± SD from one intact kidney lobule per animal (n = 3 animals per group).

3.7. Renal Corpuscle Density

Renal corpuscle density did not differ significantly between strains. No consistent temporal trend was evident from week 1 to week 8

3.8. Renal Corpuscle Diameter

Renal corpuscle diameter did not differ significantly between strains. The graph showed an inconsistent pattern with comparable values in

both strains (Figure 6B). In the Darag group, values exceeded 40 µm at weeks 2 and 6; in the Redbro group, values exceeded 40 µm at weeks 4 and 6. These observations indicate minimal between-strain differences in corpuscle lumen diameter.

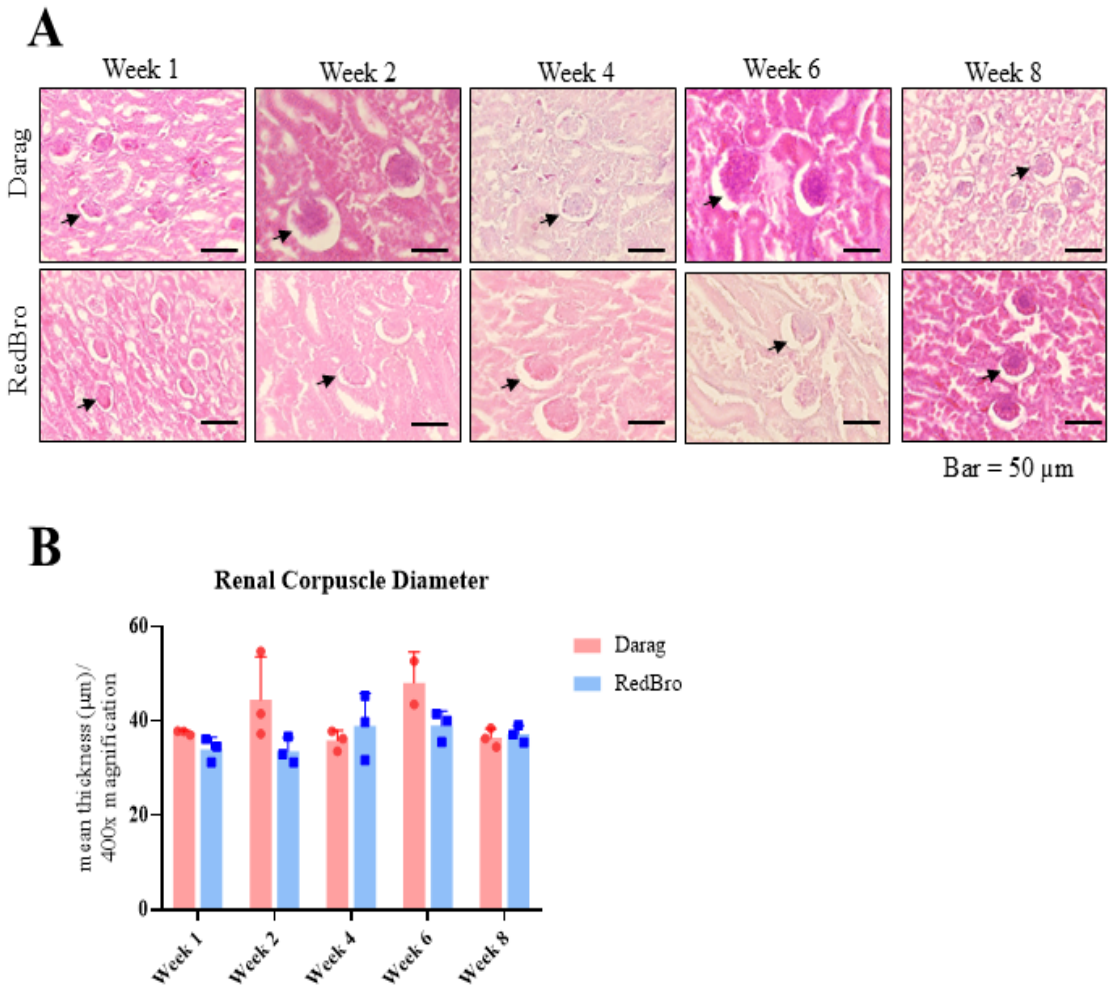

Figure 6. Histomorphological assessment of renal corpuscle diameter in Darag and Redbro strains, showing no significant differences between strains. (A) Representative images of renal corpuscles at weeks 1, 2, 4, 6, and 8; corpuscles are labeled with arrows. Scale bar = 50 µm. (B) Bar graph showing mean renal corpuscle diameter. Data are presented as mean ± SD from five corpuscles per animal (n = 3 animals per group).

3.9. Convoluted Tubule Diameters

Proximal convoluted tubule (PCT) diameter did not differ significantly between the Darag and Redbro groups (Figure 7B). No consistent pattern was observed from week 1 to week 8. The same applied to DCT diameter; Figure 7C shows an approximately stable distribution across weeks for both groups. For both PCT and DCT, the strains were comparable.

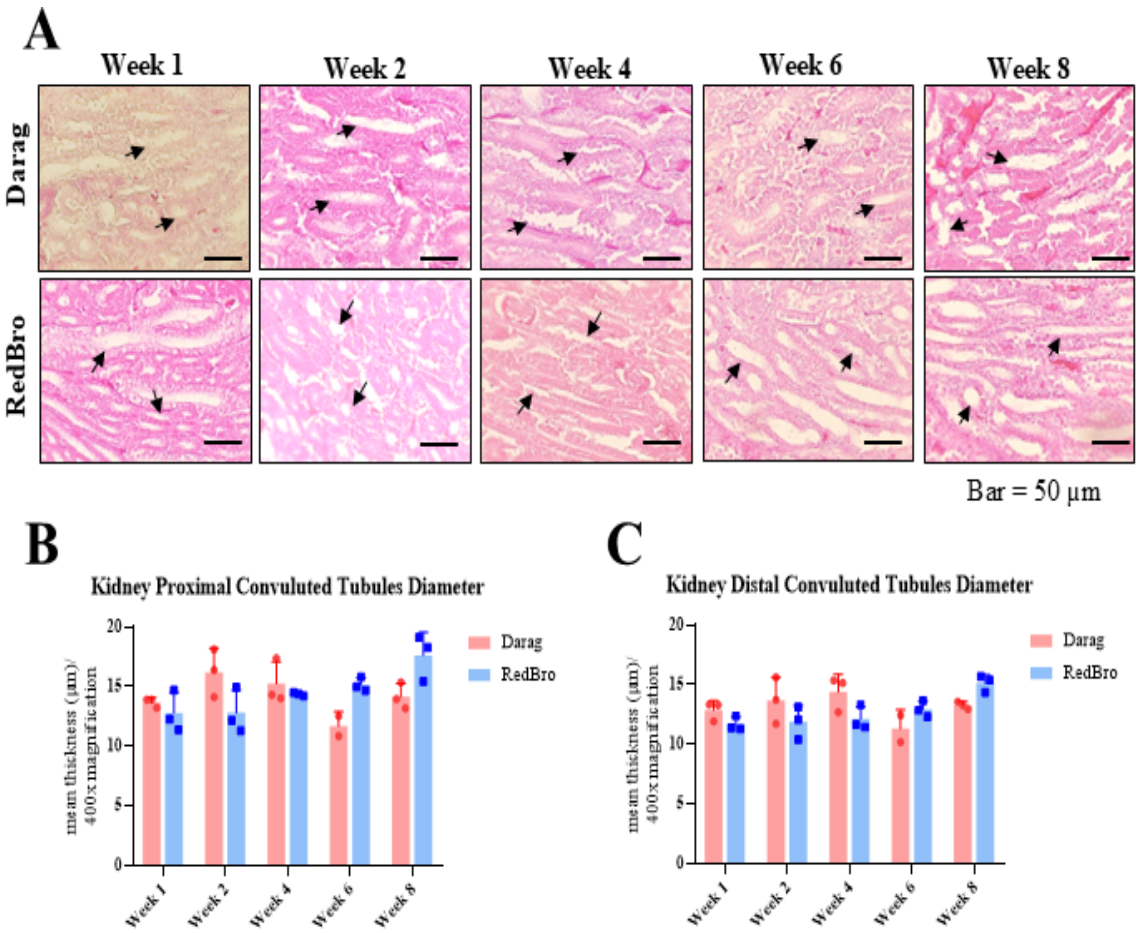

Figure 7. Histomorphology of proximal and distal convoluted tubule diameter in Darag and Redbro strains. No significant differences were observed between strains. (A) Representative kidney images at weeks 1, 2, 4, 6, and 8; proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) are labeled with arrows. Scale bar = 50 µm. (B) Bar graph showing mean PCT diameter. (C) Bar graph showing mean DCT diameter. Data are mean ± SD from five tubules per field across five fields per animal (n = 3 animals per group).

3.10. Effect of Age, Strain, and Age–Strain Interaction

Table 1 indicates significant effects of age on muscle fiber thickness, parabronchial density, and parabronchial lumen diameter. Strain significantly affected muscle fiber thickness and air capillary area fraction. The age–strain interaction significantly influenced all kidney parameters assessed (renal corpuscle density, renal corpuscle diameter, and PCT and DCT diameter).

3.11. Correlation between Heart Parameters and Body Weight

Muscle fiber thickness was the only heart parameter that correlated significantly with body BW. In both strains, BW was strongly and positively correlated with muscle fiber thickness (Darag: r = 0.97, P = 0.008; Redbro: r = 0.97, P = 0.007). Left and right ventricular wall thickness showed no significant correlation with BW in either strain (Table 2).

3.12. Correlation between Lung Parameters and Body Weight

Two lung parameters correlated with BW in both strains. In the Darag group, BW showed a strong negative correlation with parabronchial density (r = −0.94, P = 0.02) and a positive correlation with parabronchial lumen diameter (r = 0.88, P = 0.048). In the Redbro group, BW similarly showed a strong negative correlation with parabronchial density (r = −0.95, P = 0.02) and a very strong positive correlation with parabronchial lumen diameter (r = 0.996, P = 0.0004). Air capillary area fraction did not correlate with BW in either strain (Table 2).

3.13. Correlation between Kidney Parameters and Body Weight

For kidney parameters, only Redbro chickens showed significant correlations with BW. A strong positive correlation was observed between BW and PCT diameter (r = 0.996, P = 0.0003) and between BW and DCT diameter (r = 0.94, P = 0.02). Renal corpuscle density and diameter were not

Table 1. Mixed-effects model results for age and strain effects on each dependent variable.

Dependent Variable	Age	Strain	Interaction
Left ventricular wall thickness	F(1.699, 2.974) = 3.889 *P = 0.1475*	F(1, 4) = 0.2622 *P = 0.6356*	F(4, 7) = 1.505 *P = 0.2984*
Right ventricular wall thickness	F(1.256, 5.024) = 2.062 *P = 0.2155*	F(1, 16) = 1.827 *P = 0.1953*	F(4, 16) = 1.412 *P = 0.2749*
Muscle fiber thickness	F(1.968, 6.395) = 44.38 *P = 0.0002***	F(1, 4) = 28.89 *P = 0.0058***	F(4, 13) = 2.992 *P = 0.0592*
Parabronchial density	F(1.933, 6.283) = 12.70 *P = 0.0064***	F(1, 4) = 0.3086 *P = 0.6081*	F(4, 13) = 0.6921 *P = 0.6105*
Parabronchial lumen diameter	F(1.624, 5.279) = 21.78 *P = 0.0033***	F(1, 4) = 1.160 *P = 0.3421*	F(4, 13) = 1.052 *P = 0.4183*
Air capillary area fraction	F(1.168, 3.796) = 0.4199 *P = 0.5846*	F(1, 4) = 7.884 *P = 0.0484**	F(4, 13) = 2.014 *P = 0.1518*
Renal corpuscle density	F(1.167, 4.378) = 3.853 *P = 0.114*	F(1, 4) = 0.6042 *P = 0.4804*	F(4, 15) = 3.761 *P = 0.026**
Renal corpuscle diameter	F(2.298, 8.618) = 2.971 *P = 0.1003*	F(1, 4) = 2.481 *P = 0.1904*	F(4, 15) = 3.515 *P = 0.0325**
PCT diameter	F(1.649, 6.185) = 3.179 *P = 0.1161*	F(1, 4) = 0.4001 *P = 0.5614*	F(4, 15) = 6.063 *P = 0.0041***
DCT diameter	F(1.792, 6.720) = 3.178 *P = 0.1093*	F(1, 4) = 0.6806 *P = 0.4557*	F(4, 15) = 4.243 *P = 0.0171**

** P < 0.05 = significant; ** P < 0.01 = highly significant; *** P < 0.001 = very significant (two-tailed)*

Table 2. Pearson correlation coefficients between histomorphometric parameters and body weight.

Dependent Variable		Darag	Redbro
Left ventricular wall thickness	r	0.7949	0.764
	P (two-tailed)	0.108	0.1327
	N	5	5
Right ventricular wall thickness	r	0.727	0.5536
	P (two-tailed)	0.1641	0.333
	N	5	5
Muscle fiber thickness	r	0.9656	0.9676
	P (two-tailed)	0.0076**	0.007**
	N	5	5
Parabronchial density	r	−0.9448	−0.9452
	P (two-tailed)	0.0154*	0.0153*
	N	5	5
Parabronchial lumen diameter	r	0.8821	0.9955
	P (two-tailed)	0.0477*	0.0004***
	N	5	5
Air capillary area fraction	r	0.5192	−0.1892
	P (two-tailed)	0.37	0.7606
	N	5	5
Renal corpuscle density	r	−0.3887	0.6083
	P (two-tailed)	0.5178	0.2763
	N	5	5
Renal corpuscle diameter	r	−0.06011	0.6669
	P (two-tailed)	0.9235	0.2189
	N	5	5
PCT diameter	r	−0.3741	0.9964
	P (two-tailed)	0.5351	0.0003***
	N	5	5
DCT diameter	r	−0.2439	0.9408
	P (two-tailed)	0.6926	0.0172*
	N	5	5

** P < 0.05; ** P < 0.01; *** P < 0.001 (two-tailed)*

significantly correlated with BW in either strain, and PCT and DCT diameters were not significantly correlated in Darag chickens (Table 2).

3.14. Correlation between Heart Parameters and Total Feed Intake

Both strains exhibited significant correlations between heart parameters and total feed intake. In Darag chickens, total feed intake was strongly and positively correlated with muscle fiber thickness ($r = 0.98$, $P = 0.002$). In the Redbro group, left ventricular wall thickness showed a significant positive correlation ($r = 0.90$, $P = 0.04$), and muscle fiber thickness showed a highly significant positive correlation ($r = 0.98$, $P = 0.004$). Right ventricular wall thickness showed no significant correlation in either strain (Table 3).

Table 3. Pearson correlation coefficients between histomorphometric parameters and total feed intake.

Dependent Variable		Darag	Redbro
Left ventricular wall thickness	r	0.7423	0.9027
	P (two-tailed)	0.1508	0.0359*
	N	5	5
Right ventricular wall thickness	r	0.719	0.675
	P (two-tailed)	0.1711	0.2112
	N	5	5
Muscle fiber thickness	r	0.9844	0.9769
	P (two-tailed)	0.0023**	0.0042**
	N	5	5
Parabronchial density	r	−0.9713	−0.871
	P (two-tailed)	0.0058**	0.0545
	N	5	5
Parabronchial lumen diameter	r	0.932	0.94
	P (two-tailed)	0.0211*	0.0175*
	N	5	5
Air capillary area fraction	r	0.5519	−0.4354
	P (two-tailed)	0.3348	0.4637
	N	5	5
Renal corpuscle density	r	−0.44	0.3997
	P (two-tailed)	0.4584	0.505
	N	5	5
Renal corpuscle diameter	r	0.007675	0.7588
	P (two-tailed)	0.9902	0.1369
	N	5	5
PCT diameter	r	−0.3946	0.9455
	P (two-tailed)	0.5109	0.0152*
	N	5	5
DCT diameter	r	−0.261	0.8575
	P (two-tailed)	0.6715	0.0632
	N	5	5

** P < 0.05; ** P < 0.01; *** P < 0.001 (two-tailed)*

3.15. Correlation between Lung Parameters and Total Feed Intake

In the Darag group, total feed intake showed a highly significant negative correlation with parabronchial density (r = −0.97, P = 0.006) and a positive correlation with parabronchial lumen diameter (r = 0.93, P = 0.02). In the Redbro group, total feed intake showed a positive correlation with parabronchial lumen diameter (r = 0.94, P = 0.02).

3.16. Correlation between Kidney Parameters and Total Feed Intake

Redbro chickens showed a significant positive correlation between total feed intake and PCT diameter (r = 0.95, P = 0.02). In the Darag group, none of the kidney parameters were significantly correlated with total feed intake.

4. Discussion

Philippine native chicken strains such as Darag grow more slowly, typically requiring 75–120 days to reach slaughter weight [5]; consumers, particularly in rural areas, often prefer native chickens for their meat quality. Consequently, native chicken breeds are important for maintaining poultry genetic diversity and provide considerable economic value [6]. In contrast, Redbro chickens reflect years of genetic selection to improve growth rate and feed conversion efficiency [7].

Histomorphometric analysis of the heart showed that Darag chickens had thinner cardiac muscle fibers at weeks 4 and 8 than Redbro chickens (Figure 3B). Although Darag chickens are typically managed under free-range systems and Redbro chickens under intensive commercial production in field settings, both strains were maintained under identical controlled conditions in the present study. The observed between-strain differences in cardiac muscle fiber thickness may reflect inherent strain-related differences rather than management divergence. The irregular fluctuations observed in ventricular wall thickness for both strains are likely attributable to high intra-group biological variability within the small samples. Redbro demonstrated a more consistent increase in cardiac muscle fiber thickness from week 1 to week 8, whereas Darag appeared to exhibit a more gradual pattern of cardiac structural development within the sampling period. Further work at the cardiomyocyte level is needed to clarify the structural basis of these differences. It should be noted that no physiological measurements (e.g., cardiac output) were performed; the findings herein are strictly structural in nature [8,9].

Parabronchial density and lumen diameter did not differ significantly between strains. Both strains exhibited a decline in parabronchial density and an increase in lumen diameter over time (Figures 4B and 4C). These trends are attributed to apparent changes in density as the lung parenchyma expands with growth; however, organ size measurements were not incorporated into the current analysis, and this interpretation remains preliminary. Although organ weights were recorded as part of the necropsy protocol, comparative organ-to-body-weight analysis was not performed in the present study; future work should incorporate these measures. Air capillary area fraction differed significantly between strains at week 8 (Figure 4D); the structural basis for this difference remains unclear and warrants further investigation. It is acknowledged that air capillary area fraction alone does not permit inferences about gas exchange capacity without functional measurements [10]. Across more than 9,000 avian species, respiratory architecture is broadly conserved, and strain-level variation in parabronchial density or structure has been little described [11–13].

Kidney parameters showed no statistically significant between-strain differences across all measures (Figures 5–7), suggesting that Darag and Redbro chickens possess comparable renal filtration architecture at the structural level, particularly in mammalian-type renal corpuscles and proximal and distal convoluted tubules. However, the significant age × strain interaction effects observed in the mixed-effects model suggest that renal maturation trajectories may differ temporally, with each strain potentially following distinct developmental patterns before converging toward similar adult renal morphology. It is acknowledged that the avian kidney contains two structural types of renal corpuscles: the larger mammalian-type (cortical) corpuscle and the smaller reptilian-type (medullary) corpuscle. In the present study, measurements were limited to mammalian-type corpuscles, which are more readily identifiable in H&E-stained sections. Future studies should stratify measurements by corpuscle type to more comprehensively characterize renal filtration architecture in these strains. Comparable findings extended to PCT and DCT diameters, with both strains following similar trends (Figures 7B and 7C). Few studies have directly compared chicken strains, although notable interspecies differences in renal structure have been reported [14].

The mixed-effects model showed that age had a very significant effect on heart muscle fiber thickness, reflecting cardiac structural growth. Age also had highly significant effects on parabronchial density and lumen diameter; parabronchi decreased in apparent number as lungs grew, whereas lumen diameter increased. Strain effects were observed for muscle fiber thickness and air capillary area fraction; Redbro chickens had thicker muscle fibers, and Darag

chickens had a higher air capillary area fraction at week 8. Significant age–strain interactions were detected for all kidney parameters, indicating that renal maturation trajectories, while ultimately converging, may differ temporally between strains.

Body weight correlated strongly and positively with muscle fiber thickness in both strains, consistent with somatic growth. Parabronchial density correlated negatively with body weight in both strains, likely because fewer parabronchi are captured within a fixed microscopic field as lungs enlarge. In the Redbro group, parabronchial lumen diameter showed a very strong positive correlation with body weight; in the Darag group, the correlation was positive but more modest, consistent with a proportional growth pattern [15]. For kidney parameters, PCT and DCT diameters showed significant positive correlations with body weight only in Redbro chickens. Because this pattern was absent in the Darag group, a strain-specific developmental difference may exist, although this interpretation remains speculative given the small sample size. Reported effects of glucocorticoid growth promoters on broiler renal tubule morphology may provide context for these observations, but their contribution here remains to be investigated [16].

Analysis of correlations between total feed intake and histomorphometric parameters identified several significant associations consistent with those for body weight. Feed intake is a primary driver of poultry growth, development, and production [17,18]. The Redbro group consumed more than twice as much feed as the Darag group over the study period. Pelleted diets used in commercial broiler production are designed to improve feed efficiency and intake [19]; genetic and management differences between commercial broilers and native strains are also relevant [20].

Overall, the results provide preliminary evidence of between-strain differences in selected parameters, whereas most parameters were comparable between Darag and Redbro under the controlled experimental conditions of this study.

5. Conclusions

This study compared the histomorphology of the heart, lungs, and kidneys of the Philippine native Darag chicken strain with those of commercial hybrid Redbro chickens to characterize between-strain differences. The findings provide preliminary structural baseline data on Darag cardiopulmonary and renal histomorphometry and identify parameters where the strain is comparable to its slow-growing broiler counterpart under controlled conditions.

The present study is exploratory given the small number of animals per sampling period (three per group per time point), and all findings should be regarded as indicative trends pending confirmation in larger cohorts. No physiological measurements were performed; conclusions are limited to structural observations. The irregular trends observed in several parameters likely reflect high intra-group biological variability inherent to the sample size used. A formal inter-observer reliability assessment was not conducted and is acknowledged as a limitation.

Specific areas for future investigation include: incorporating organ-to-body-weight ratios and organ size measurements; distinguishing mammalian-type from reptilian-type renal corpuscles in morphometric analyses; increasing sample sizes to improve statistical power; and examining additional Philippine native chicken strains such as ZamPen, Caraga Black [21,22], Paroakan, Bolinao, Banaba, and Joloano [23]. Sample processing precision should be ensured to minimize slide artifacts. These collective efforts will clarify the histomorphometric traits of native Philippine chicken strains and expand baseline anatomical data for future comparative and functional studies.

Ethics Approval and Consent to Participate

All procedures were approved by the University of the Philippines Los Baños Institutional Animal Care and Use Committee (approval number UPLB-2023-036).

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Tomacruz, S. (2017, April 14). *Fast facts: How much meat does the Filipino consume?* Rappler. https://www.rappler.com/business/166870-fast-facts-meat-chicken-pork-filipino-consumption/.

[2] Philippine Statistics Authority. (2018). *2018 annual report*. https://psa.gov.ph/.

[3] Deng, S., Xing, T., Li, C., Xu, X., & Zhou, G. (2022). The effect of breed and age on growth performance, carcass traits, and metabolic profile in breast muscle of Chinese indigenous chickens. *Foods, 11*(3), 483. https://doi.org/10.3390/foods11030483.

[4] Contreras, R.C.C., Catamin, R.D., Paragados, D.A., & De La Cruz, A.C. (2014). Acceptability of native Darag chicken menu variations. *Asia Pacific Journal of Multidisciplinary Research, 2*(3), 12–17. https://ejournals.ph/article.php?id=5774.

[5] Department of Trade and Industry, & Philippine Council for Agriculture, Forestry and Natural Resources Research and Development. (2009). *The "Darag" native chicken.*

[6] Moharrery, A., & Mirzaei, M. (2014). Growth characteristics of commercial broiler and native chickens as predicted by different growth functions. *Journal of Animal and Feed Sciences, 23*(1), 82–89. https://doi.org/10.22358/jafs/65720/2014.

[7] Huerta, A., Pascual, A., Bordignon, F., Trocino, A., Xiccato, G., Cartoni Mancinelli, A., Mugnai, C., Pirrone, F., & Birolo, M. (2023). Resiliency of fast-growing and slow-growing genotypes of broiler chickens submitted to different environmental temperatures: Growth performance and meat quality. *Poultry Science, 102*(12), 103158. https://doi.org/10.1016/j.psj.2023.103158.

[8] Dzialowski, E. M., & Crossley, D. A. (2015). The cardiovascular system. In C. G. Scanes (ed.), *Sturkie's Avian Physiology*. 6th ed. pp. 193–283. Academic Press: USA.

[9] Gesek, M., Otrocka-Domagała, I., Sokół, R., Paździor-Czapula, K., Lambert, B.D., Wiśniewska, A.M., Żechowicz, M., Mikiewicz, M., & Korzeniowska, P. (2016). Histopathological studies of the heart in three lines of broiler chickens. *British Poultry Science, 57*(2), 219–226. https://doi.org/10.1080/00071668.2016.1154505.

[10] West, J. B., Fu, Z., Deerinck, T.J., Mackey, M.R., Obayashi, J.T., & Ellisman, M.H. (2010). Structure–function studies of blood and air capillaries in chicken lung using 3D electron microscopy. *Respiratory Physiology & Neurobiology, 170*(2), 202–209. https://doi.org/10.1016/j.resp.2009.12.010.

[11] Kirbaş, G., & Takçı, I. (2018). Anatomy of respiratory system in poultry. *Mehmet Akif Ersoy Üniversitesi Veteriner Fakültesi Dergisi, 3*(2), 141–147. https://doi.org/10.24880/maeuvfd.433946.

[12] Maina, J.N. (2017). Pivotal debates and controversies on the structure and function of the avian respiratory system. *Biological Reviews, 92*(3), 1475–1504. https://doi.org/10.1111/brv.12292.

[13] Maina, J.N. (2015). The design of the avian respiratory system: Development, morphology and function. *Journal of Ornithology, 156*(1), 41–63. https://doi.org/10.1007/s10336-015-1263-9.

[14] Fatahian Dehkordi, R.A., & Habibi, A. (2015). Stereological and electron microscopic study on the internal organization of the urinary secretory units in three species of birds. *Journal of Applied Animal Research, 43*(1), 118–124. https://doi.org/10.1080/09712119.2014.928625.

[15] Nascimento, S.T., Silva, I.J.O.D., Mourão, G.B., & Castro, A.C.D. (2012). Bands of respiratory rate and cloacal temperature for different broiler chicken strains. *Revista Brasileira de Zootecnia, 41*(5), 1318–1324. https://doi.org/10.1590/S1516-35982012000500033.

[16] Sultana, N., Islam, R., Akter, A., Ayman, U., Bhakta, S., Rony, S.A., Nahar, A., & Alam, R. (2021). Biochemical and morphological attributes of broiler kidney in response to dietary glucocorticoid, dexamethasone. *Saudi Journal of Biological Sciences, 28*(12), 6721–6729. https://doi.org/10.1016/j.sjbs.2021.07.047.

[17] Abdollahi, M.R., Zaefarian, F., & Ravindran, V. (2018). Feed intake response of broilers: Impact of feed processing. *Animal Feed Science and Technology, 237*, 154–165. https://doi.org/10.1016/j.anifeedsci.2017.12.013.

[18] Wang, J., Liu, Z., Lin, H., Jiao, H., Zhao, J., Ma, B., Wang, Y., He, S., & Wang, X. (2024). Daily feeding frequency affects feed intake and body weight management of growing layers. *Poultry Science, 103*(6), 103748. https://doi.org/10.1016/j.psj.2024.103748.

[19] Abdollahi, M.R., Ravindran, V., & Svihus, B. (2013). Pelleting of broiler diets: An overview with emphasis on pellet quality and nutritional value. *Animal Feed Science and Technology, 179*(1), 1–23. https://doi.org/10.1016/j.anifeedsci.2012.11.002.

[20] Sagcal, J. (2022, May 2). *Breeding and caring for Darag native chickens.* Manila Bulletin. https://mb.com.ph/2022/05/02/breeding-and-caring-for-darag-native-chickens/.

[21] DOST-PCAARRD. (2022a). *Development of Caraga black native chicken through selection and breeding as potential niche product of Caraga Region.* https://ispweb.pcaarrd.dost.gov.ph/.

[22] DOST-PCAARRD. (2022b). *Establishment of Zampen native chicken breeding population with improved egg production and growth performance.* https://ispweb.pcaarrd.dost.gov.ph/.

[23] Yan, G. (2020, August 14). *Farming heritage chicken breeds of the Philippines.* The Poultry Site. https://www.thepoultrysite.com/articles/farming-heritage-chickenbreeds-of-the philippines.